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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 333/34, C07C 259/06, A61K 31/38, 31/185 — A1

(11) International Publication Number: WO 97/26257

(43) International Publication Date: 24 July 1997 (24.07.97)

(21) International Application Number: PCT/EP97/00196

(22) International Filing Date: 14 January 1997 (14.01.97)

(30) Priority Data:
9601041.8 17 January 1996 (17.01.96) GB

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

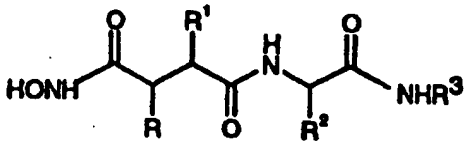
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS

(57) Abstract

Compounds of formula (I) wherein R and R^3 are each independently hydrogen, alkyl, alkenyl, alkynyl or aryl; R^1 is arylmethyl; and R^2 is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl, and pharmaceutically acceptable derivatives thereof, are useful in the treatment and prophylaxis of disorders in which the overproduction of sCD23 is implicated.



(I)

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HYDROXAMIC ACID BASED COLLAGENASE INHIBITORS.

This invention relates to novel inhibitors of the formation of soluble human CD23 and their use in the treatment of conditions associated with excess production of soluble CD23 (s-CD23) such as autoimmune disease and allergy.

CD23 (the low affinity IgE receptor FceRII, Blast 2), is a 45 kDa type II integral protein expressed on the surface of a variety of mature cells, including B and T lymphocytes, macrophages, natural killer cells, Langerhans cells, monocytes and platelets (Delespesse *et al, Adv Immunol,* 49 [1991] 149-191). There is also a CD23-like molecule on eosinophils (Grangette *et al, J Immunol,* 143 [1989] 3580-3588). CD23 has been implicated in the regulation of the immune response (Delespesse *et al, Immunol Rev,* 125 [1992] 77-97). Human CD23 exists as two differentially regulated isoforms, a and b, which differ only in the amino acids at the intracellular N-terminus (Yokota *et al, Cell,* 55 [1988] 611-618). In man the constitutive a isoform is found only on B-lymphocytes, whereas type b, inducible by IL4, is found on all cells capable of expressing CD23.

Intact, cell bound CD23 (i-CD23) is known to undergo cleavage from the cell surface leading to the formation of a number of well-defined soluble fragments (s-CD23), which are produced as a result of a complex sequence of proteolytic events, the mechanism of which is still poorly understood (Bourget *et al J Biol Chem,* 269 [1994] 6927-6930). Although not yet proven, it is postulated that the major soluble fragments (Mr 37, 33, 29 and 25 kDa) of these proteolytic events, all of which retain the C-terminal lectin domain common to i-CD23, occur sequentially via initial formation of the 37 kDa fragment (Letellier *et al, J Exp Med,* 172 [1990] 693-700). An alternative intracellular cleavage pathway leads to a stable 16 kDa fragment differing in the C-terminal domain from i-CD23 (Grenier-Brosette *et al, Eur J Immunol,* 22 [1992] 1573-1577).

Several activities have been ascribed to membrane bound i-CD23 in humans, all of which have been shown to play a role in IgE regulation. Particular activities include: a) antigen presentation, b) IgE mediated eosinophil cytotoxicity, c) B cell homing to germinal centres of lymph nodes and spleen, and d) downregulation of IgE synthesis (Delespesse *et al, Adv Immunol,* 49, [1991] 149-191). The three higher molecular weight soluble CD23 fragments (Mr 37, 33 and 29 kDa) have multifunctional cytokine properties which appear to play a major role in IgE production. Thus, the excessive formation of s-CD23 has been

implicated in the overproduction of IgE, the hallmark of allergic diseases such as extrinsic asthma, rhinitis, allergic conjuctivitis, eczema, atopic dermatitis and anaphylaxis (Sutton and Gould, *Nature*, 366, [1993] 421-428).

Other biological activities attributed to s-CD23 include the stimulation of B cell growth and the induction of the release of mediators from monocytes. Thus, elevated levels of s-CD23 have been observed in the serum of patients having B-chronic lymphocytic leukaemia (Sarfati *et al, Blood*, 71 [1988] 94-98) and in the synovial fluids of patients with rheumatoid arthritis (Chomarat *et al, Arthritis and Rheumatism*, 36 [1993] 234-242). That there is a role for CD23 in inflammation is suggested by a number of sources. First, sCD23 has been reported to bind to extracellular receptors which when activated are involved in cell-mediated events of inflammation. Thus, sCD23 is reported to directly activate monocyte TNF, IL-1, and IL-6 release (Armant et al, vol 180, J.Exp. Med., 1005-1011 (1994)). CD23 has been reported to interact with the B2-integrin adhesion molecules, CD11b and CD11c on monocyte/macrophage (S. Lecoanet-Henchoz et al, Immunity, vol 3; 119-125 (1995)) which trigger $NO2^-$, hydrogen peroxide and cytokine (IL-1, IL-6, and TNF) release. Finally, IL-4 or IFN induce the expression of CD23 and its release as sCD23 by human monocytes. Ligation of the membrane bound CD23 receptor with IgE/anti-IgE immune complexes or anti CD23 mAb activates cAMP and IL-6 production and thromboxane B2 formation, demonstrating a receptor-mediated role of CD23 in inflammation.

Because of these various properties of CD23, compounds which inhibit the formation of s-CD23 should have twofold actions of a) enhancing negative feedback inhibition of IgE synthesis by maintaining levels of i-CD23 on the surface of B cells, and b) inhibiting the immunostimulatory cytokine activities of higher molecular weight soluble fragments (Mr 37, 33 and 29 kDa) of s-CD23. In addition, inhibition of CD23 cleavage should mitigate sCD23-induced monocyte activation and mediator formation, thereby reducing the inflammatory response.

International Patent Application No. PCT/EP95/02693 (Smithkline Beecham plc) discloses that compounds which inhibit the action of matrix metalloproteases (eg collagenase, stromelysin and gelatinase) are effective inhibitors of the release of human soluble CD23 transfected into mammalian cell culture systems. Known inhibitors of matrix metalloprotease include the compounds described in the patent publications listed in the Table.

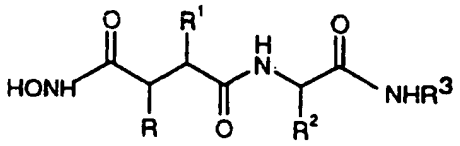
TABLE

Patent publication	Compounds disclosed	Specific compounds and methods of preparation- Example Nos.
US-A-4,595,700	Compounds of formula (I) as defined in claim 1, optionally as further subdefined in the description.	1 to 8.
US-A-4,599,361		1 to 7.
GB-A-2 268 934		1 to 10.
GB-A-2 272 441		1 to 5.
EP-A-0 231 081		1 to 8.
EP-A-0 236 872		1 to 28.
EP-A-0 262 053		1 to 15.
EP-A-0 273 689		1 to 38.
EP-A-0 276 436		1 to 44.
EP-A-0 274 453		1 to 8.
EP-A-0 320 118		1 to 5.
EP-A-0 489 577		1 to 25.
EP-A-0 489 579		1 to 4.
EP-A-0 497 192		1 to 80.
EP-A-0 498 665		1 to 27.
EP-A-0 520 573		1 to 34.
EP-A-0 574 758		1 to 43.
EP-A-0 575 844		1 to 27.
EP-A-0 606 046		1 to 32.
EP-A-0 613 883		1 to 7.
EP-A-0 621 270		1 to 40.
WO 90/05716		1 to 38.
WO 90/05719		1 to 26.
WO 91/02716		1 to 17.
WO 92/09563	Compounds of formula (1) or (2) as defined in claim 1.	1 to 21.

TABLE contd.

Patent publication	Compounds disclosed	Specific compounds and methods of preparation- Example Nos.
WO 92/13831	Compounds of formula (I) as defined in claim 1, optionally as further subdefined in the description.	1 to 27.
WO 92/21360		1 to 5.
WO 92/22523		I to X.
WO 93/14096		1 to 8.
WO 93/20047		1 to 14.
WO 93/24475		1 to 6.
WO 93/24449		1 to 8.
WO 94/00119		1 to 86.
WO 94/07481		1 to 15.
WO 94/12169		1 to 24.
WO 94/21625		1 to 7.
WO 94/21612		1 to 116.
WO 94/24140		1 to 5.
WO 94/25434		1 to 7.
WO 94/25435		Example 1.
WO 95/04033		Examples 1 to 7.
WO 95/04715		All examples.
WO 95/12603		All examples.

According to the present invention, there is provided a compound of formula (I):



(I)

wherein R and R3 are each independently hydrogen, alkyl, alkenyl, alkynyl or aryl; R1 is arylmethyl; and R2 is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl.

Alkyl, alkenyl and alkynyl groups referred to herein include straight and branched groups containing up to six carbon atoms and are optionally substituted by one or more groups selected from the group consisting of aryl, heterocyclyl, (C_{1-6})alkylthio, (C_{1-6})alkenylthio, (C_{1-6})alkynylthio, arylthio, heterocyclylthio, (C_{1-6})alkoxy, aryl(C_{1-6})alkoxy, aryl(C_{1-6})alkylthio, amino, mono- or di-(C_{1-6})alkylamino, cycloalkyl, cycloalkenyl, carboxy and esters thereof, hydroxy, and halogen.

Cycloalkyl and cycloalkenyl groups referred to herein include groups having between three and eight ring carbon atoms and are optionally substituted as described hereinabove for alkyl, alkenyl and alkynyl groups.

When used herein, the term "aryl" includes phenyl and naphthyl such as 2-naphthyl. Suitably any aryl group, including phenyl and naphthyl, may be optionally substituted by up to five, preferably up to three substituents. Suitable substituents include halogen, (C_{1-6})alkyl, aryl(C_{1-6})alkyl, (C_{1-6})alkoxy, (C_{1-6})alkoxy(C_{1-6})alkyl, halo(C_{1-6})alkyl, hydroxy, nitro, amino, mono- and di-*N*-(C_{1-6})alkylamino, acylamino, acyloxy, carboxy, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C_{1-6})alkylcarbamoyl, (C_{1-6})alkoxycarbonyl, aryloxycarbonyl, ureido, guanidino, sulphonylamino, aminosulphonyl, (C_{1-6})alkylthio, (C_{1-6})alkyl sulphinyl (C_{1-6})alkylsulphonyl, heterocyclyl and heterocyclyl (C_{1-6})alkyl. In addition, two adjacent ring carbon atoms may be linked by a (C_{3-5})alkylene chain, to form a carbocyclic ring.

When used herein the terms "heterocyclyl" and "heterocyclic" suitably include, unless otherwise defined, aromatic and non-aromatic, single and fused, rings suitably containing up to four heteroatoms in each ring, each of which is selected from oxygen, nitrogen and sulphur, which rings, may be unsubstituted or substituted by, for example, up to three

substituents. Each heterocyclic ring suitably has from 4 to 7, preferably 5 or 6, ring atoms. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring.

Preferably a substituent for a heterocyclyl group is selected from halogen, (C_{1-6})alkyl, aryl(C_{1-6})alkyl, (C_{1-6})alkoxy, (C_{1-6})alkoxy(C_{1-6})alkyl, halo(C_{1-6})alkyl, hydroxy, amino, mono- and di-*N*-(C_{1-6})alkyl-amino, acylamino, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C_{1-6})alkylcarbonyl, aryloxycarbonyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, aryl, oxy groups, ureido, guanidino, sulphonylamino, aminosulphonyl, (C_{1-6})alkylthio, (C_{1-6})alkylsulphinyl, (C_{1-6})alkylsulphonyl, heterocyclyl and heterocyclyl(C_{1-6})alkyl.

In a particular aspect of the invention, R is hydrogen or methyl, optionally substituted by arylthio or heterocyclylthio; and/or R1 is a benzyl group; and/or R2 is a benzyl group; and/or R3 is hydrogen, methyl or benzyl.

According to a further aspect, the present invention provides the use of a compound of formula (I) for the production of a medicament for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders, and autoimmune disease, in which the overproduction of s-CD23 is implicated.

In a further aspect the invention provides a method for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders, and autoimmune disease, in which the overproduction of s-CD23 is implicated, which method comprises the administration of a compound of formula (I), to a human or non-human mammal in need thereof.

The invention also provides a pharmaceutical composition for the treatment or prophylaxis of disorders such as allergy, inflammatory disorders, and autoimmune disease, in which the overproduction of s-CD23 is implicated which comprises a compound of formula (I) and optionally a pharmaceutically acceptable carrier therefor.

Particular inflammatory disorders include CNS disorders such as Alzheimers disease, multiple sclerosis, and multi-infarct dementia, as well as the inflammation mediated sequelae of stroke and head trauma.

It is to be understood that the pharmaceutically acceptable salts, solvates and other pharmaceutically acceptable derivatives of the compound of formula (I) are also included in the present invention.

Salts of compounds of formula (I) include for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, hydroiodides, p-toluenesulphonates, phosphates, sulphates, acetates, trifluoroacetates, propionates, citrates, maleates, fumarates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

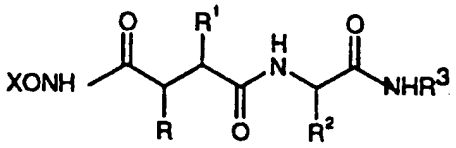
Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases, for example alkali metal salts such as sodium or potassium salts, and organic amine salts such as morpholine, piperidine, dimethylamine or diethylamine salts.

It has surprisingly been found that the compounds of the present invention are potent and selective inhibitors of CD23 processing, whilst exhibiting reduced collagenase inhibitory activity in comparison with the above-mentioned compounds of the prior art.

The compounds of the invention may be prepared by use of any appropriate conventional method, for example by analogy with the methods disclosed in patent publications WO 90/05716, WO 93/24475, WO 94/21625, WO 95/19956, WO 90/05719, WO 91/02716, WO 92/13831, WO 93/20047, EP-A-0214639, EP-A-0236872, EP-A-0274453, EP-A-0489577, EP-A-0489579, EP-A-0497192, EP-A-0574758 and USP 4599361.

Accordingly, a further aspect of the invention provides a process for preparing a compound of formula (I) as defined hereinabove, which process comprises:

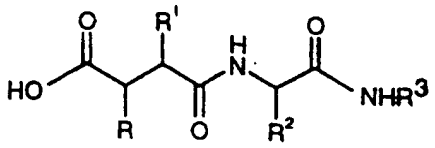
(a) deprotecting a compound of formula (II):



(II)

wherein R to R3 are as defined hereinabove, and X is a protecting group such as benzyl or trimethylsilyl or

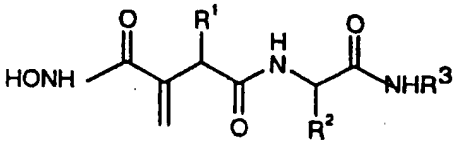
(b) reacting a compound of formula (III):



(III)

wherein R to R3 are as defined hereinabove, with hydroxylamine or a salt thereof, or

(c) reacting a compound of formula (IV):



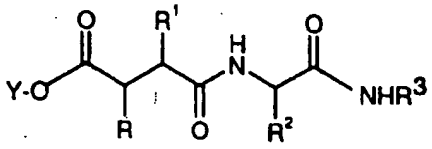
(IV)

wherein R1 to R3 are as defined hereinabove, with a thiol to give a compound of formula (I) wherein R is methyl substituted by alkylthio, arylthio, aralkylthio, or heterocyclylthio, or

(d) converting a compound of formula (I) to a different compound of formula (I) as defined hereinabove.

Compounds of formulae (II), (III) and (IV) are novel and form a further aspect of the invention.

Compounds of formula (II) can be prepared from compounds of formula (III) by reaction with a protected hydroxylamine. Compounds of formula (III) can be prepared by hydrolysis of a compound of formula (V):



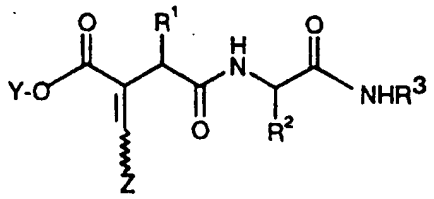
(V)

wherein R to R3 are as defined hereinabove, and Y is a protecting group such as t-butyl.

Suitable protecting groups for a hydroxamic acid are well known in the art and include benzyl, trimethylsilyl, t-butyl and t-butyldimethylsilyl.

Suitable protecting groups for a carboxylic acid are well known in the art and include t-butyl , benzyl and methyl.

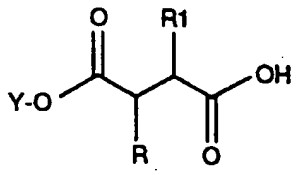
Compounds of formula (V) can be prepared by reduction of a compound of formula (VI):



(VI)

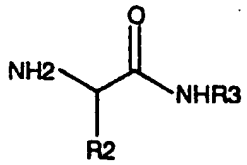
wherein R1 to R3 and Y are as defined hereinabove, and Z is a group such that ZCH_2- is R.

Compounds of formula (V) can also be prepared by reacting a compound of formula (VII):



(VII)

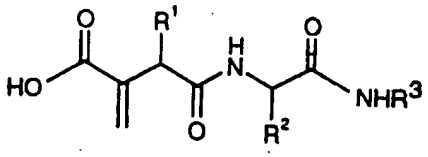
wherein R, R1 and Y are as defined hereinabove, with a compound of formula (VIII):



(VIII)

wherein R2 and R3 are as defined hereinabove, or an activated derivative thereof.

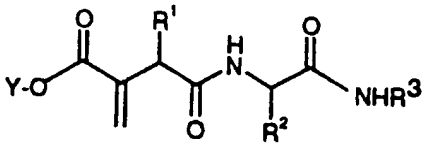
Compounds of formula (III) can also be prepared by reacting a compound of formula (IX):



(IX)

wherein R1 to R3 are as hereinabove defined, with a thiol to give a compound of formula (III) wherein R is methyl substituted by alkylthio, arylthio, aralkylthio, or heterocyclylthio.

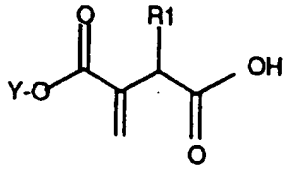
Compounds of formula (IX) can be prepared by hydrolysis of a compound of formula (X):



(X)

wherein R1 to R3 and Y are as defined hereinabove.

Compounds of formula (X) can be prepared by reacting a compound of formula (XI):



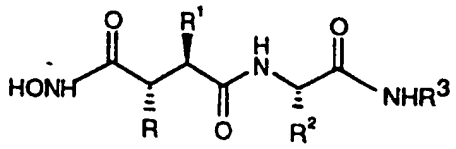
(XI)

wherein R1 and Y are as defined hereinabove, with a compound of formula (VIII) as defined hereinabove, or an activated derivative thereof.

The starting materials and other reagents are available commercially or can be synthesised by well-known and conventional methods.

The isomers, including stereoisomers, of the compounds of the present invention may be prepared as mixtures of such isomers or as individual isomers. The individual

isomers may be prepared by any appropriate method, for example individual stereoisomers may be prepared by stereospecific chemical synthesis starting from chiral substrates or by separating mixtures of diastereoisomers using known methods. In a preferred aspect, the invention provides compounds of formula (IA):



(1A)

It is preferred that the compounds are isolated in substantially pure form.

As stated herein an inhibitor of the formation of soluble human CD23 has useful medical properties. Preferably the active compounds are administered as pharmaceutically acceptable compositions.

The compositions are preferably adapted for oral administration. However, they may be adapted for other modes of administration, for example in the form of a spray, aerosol or other conventional method for inhalation, for treating respiratory tract disorders; or parenteral administration for patients suffering from heart failure. Other alternative modes of administration include sublingual or transdermal administration.

The compositions may be in the form of tablets, capsules, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

In order to obtain consistency of administration it is preferred that a composition of the invention is in the form of a unit dose.

Unit dose presentation forms for oral administration may be tablets and capsules and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

The solid oral compositions may be prepared by conventional methods of blending, filling or tabletting. Repeated blending operations may be used to distribute

the active agent throughout those compositions employing large quantities of fillers. Such operations are of course conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

Oral liquid preparations may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, and, depending on the concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, a preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

Compositions of this invention may also suitably be presented for administration to the respiratory tract as a snuff or an aerosol or solution for a nebulizer, or as a microfine powder for insufflation, alone or in combination with an inert carrier such as lactose. In such a case the particles of active compound suitably have diameters of less

than 50 microns, preferably less than 10 microns for example diameters in the range of 1-50 microns, 1-10 microns or 1-5 microns. Where appropriate, small amounts of other anti-asthmatics and bronchodilators, for example sympathomimetic amines such as isoprenaline, isoetharine, salbutamol, phenylephrine and ephedrine; xanthine derivatives such as theophylline and aminophylline and corticosteroids such as prednisolone and adrenal stimulants such as ACTH may be included.

The compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the active material, depending upon the method of administration. A preferred range for inhaled administration is 10-99%, especially 60-99%, for example 90, 95 or 99%.

Microfine powder formulations may suitably be administered in an aerosol as a metered dose or by means of a suitable breath-activated device.

Suitable metered dose aerosol formulations comprise conventional propellants, cosolvents, such as ethanol, surfactants such as oleyl alcohol, lubricants such as oleyl alcohol, desiccants such as calcium sulphate and density modifiers such as sodium chloride.

Suitable solutions for a nebulizer are isotonic sterilised solutions, optionally buffered, at for example between pH 4-7, containing up to 20mg/ml of compound but more generally 0.1 to 10mg/ml, for use with standard nebulisation equipment.

An effective amount will depend on the relative efficacy of the compounds of the present invention, the severity of the disorder being treated and the weight of the sufferer. Suitably, a unit dose form of a composition of the invention may contain from 0.1 to 1000mg of a compound of the invention (0.001 to 10mg via inhalation) and more usually from 1 to 500mg, for example 1 to 25 or 5 to 500mg. Such compositions may be administered from 1 to 6 times a day, more usually from 2 to 4 times a day, in a manner such that the daily dose is from 1mg to 1g for a 70 kg human adult and more particularly from 5 to 500mg. That is in the range of about 1.4×10^{-2} mg/kg/day to 14 mg/kg/day and more particularly in the range of about 7×10^{-2} mg/kg/day to 7 mg/kg/day.

The following examples illustrate the invention but do not limit it in any way.

BIOLOGICAL TEST METHODS

Procedure 1: The ability of test compounds to inhibit the release of soluble CD23 was investigated by use of the following procedure.

RPMI 8866 Cell membrane CD23 cleavage activity assay:

Plasma membranes from RPMI 8866 cells, a human Epstein-Barr virus transformed B-cell line (Sarfati et al., Immunology 60 [1987] 539-547) expressing high levels of CD23 are purified using an aqueous extraction method. Cells resuspended in homogenization buffer (20mM HEPES pH 7.4, 150 mM NaCl, 1.5 mM MgCl2, 1 mM DTT) are broken by N_2 cavitation in a Parr bomb and the plasma membrane fraction mixed with other membranes is recovered by centrifugation at 10,000Xg. The light pellet is resuspended in 0.2 M potassium phosphate, pH 7.2 using 2 ml per 1-3 g wet cells and the nuclear pellet is discarded. The membranes are further fractionated by partitioning between Dextran 500 (6.4% w/w) and polyethylene glycol (PEG) 5000 (6.4% w/w) (ref), at 0.25 M sucrose in a total of 16 g per 10-15 mg membrane proteins [Morre and Morre, BioTechniques 7, 946-957 (1989)]. The phases are separated by brief centrifugation at 1000Xg and the PEG (upper) phase is collected, diluted 3-5 fold with 20 mM potassium phosphate buffer pH 7.4, and centrifuged at 100,000Xg to recover membranes in that phase. The pellet is resuspended in phosphate-buffered saline and consists of 3-4 fold enriched plasma membranes as well as some other cell membranes (e.g. lysosomes, Golgi). The membranes are aliquoted and stored at -80°C. Fractionation at 6.6 % Dextran/PEG yields plasma membranes enriched 10-fold.

The fractionated membranes are incubated at 37°C for times up to 4 hrs to produce fragments of CD23 which are separated from the membrane by filtration in 0.2 micron Durapore filter plates (Millipore) after quenching the assay with 5 uM Preparation 1 from P 30994. sCD23 released from the membrane is determined using the EIA kit from The Binding Site (Birmingham, UK) or a similar one utilizing MHM6 anti-CD23 mAb [Rowe et al., Int. J. Cancer, 29, 373-382 (1982)] or another anti-CD23 mAb as the capture antibody in a sandwich EIA.. The amount of soluble CD23 made by 0.5 ug membrane protein in a total

volume of 50 ul phosphate-buffered saline is measured by EIA and compared to the amount made in the presence of various concentrations of inhibitors. Inhibitors are prepared in solutions of water or dimethylsulfoxide (DMSO) and the final DMSO concentration is not more than 2 %. IC50's are determined by curve fitting as the concentration where 50 % inhibition of production of sCD23 is observed relative to the difference in sCD23 between controls incubated without inhibitor.

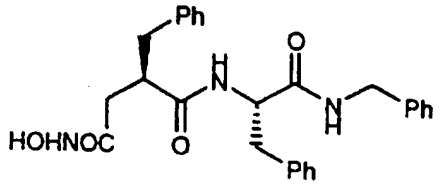
Procedure 2: The ability of test compounds to inhibit collagenase was investigated using the following procedure.

Collagenase inhibition assay:

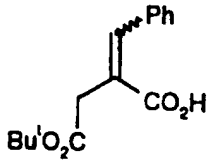
The potency of compounds to act as inhibitors of collagenase was determined by the method of Cawston and Barrett (Anal. Biochem. **99**, 340-345, 1979), hereby incorporated by reference, whereby a 1 mM solution of the inhibitor being tested or dilutions thereof, was incubated at 37 ^{o}C for 18 h with collagen and human recombinant collagenase, from synovial fibroblasts cloned, expressed and purified from E. Coli, (buffered with 150 mM Tris, pH 7.6, containing 15 mM calcium chloride, 0.05% Brij 35, 200 mM sodium chloride and 0.02% sodium azide). The collagen was acetylated ^{3}H type 1 bovine collagen prepared by the method of Cawston and Murphy (methods in Enzymology 80, 711,1981) The samples were centrifuged to sediment undigested collagen and an aliquot of the radioactive supernatant removed for assay on a scintillation counter as a measure of hydrolysis. The collagenase activity in the presence of 1mM inhibitor, or dilution thereof, was compared to activity in a control devoid of inhibitor and the results reported as that concentration effecting 50% of the collagenase (IC_{50}).

Example 1

N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-benzylamide



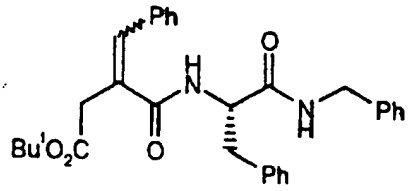
a) 2-Benzylidene-4-tert-butoxysuccinic acid



A solution of methyl 2-benzylidene-4-tert-butoxysuccinate (2g,7.25mmol) in methanol (20ml) was treated with a solution of sodium hydroxide (2M,7.25ml,14.5mmol) and the mixture stirred for 16h at 20°C. The reaction was concentrated under vacuum and water (20ml) added.The aqueous solution was extracted with ether (2x20ml), acidified with 2M hydrochloric acid and extracted again with ether (3x25ml). The ether from acidic extraction was dried ($MgSO_4$), filtered and evaporated to give the acid as a white solid (1.74g,92%). mp 110-115°C.

d_H ($CDCl_3$) 1.46 (9H,s), 3.47 (2H,s), 7.38 (5H,m), 7.94 (1H,s), 12.58,br s).

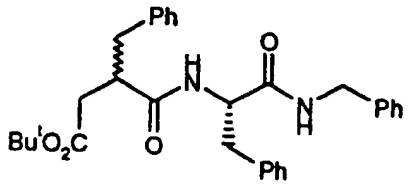
b) N-(2-Benzylidene-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-benzylamide



A solution of 2-benzylidene-4-tert-butoxysuccinic acid (524mg,2mmol), (S)-phenylalanine-N'-benzylamide hydrochloride (580mg,2mmol), 1-hydroxybenzotriazole hydrate (486mg,3.6mmol) and diisopropylethylamine (0.35ml,2mmol) in DMF (20ml) was treated at 0°C with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (424mg,2.2mmol) and the reaction stirred for 1h at 0°C and 16h at 20°C. Following evaporation, the residue was partitioned between saturated sodium hydrogen carbonate (25ml) and dichloromethane (50ml). The organic layer was washed with sodium hydrogen carbonate (2x25ml), 10% citric acid (2x25ml) and brine (25ml) and dried ($MgSO_4$). Filtration and evaporation gave a residue which was chromatographed on silica (chloroform-methanol 100:1) to give the title compound as an off-white foam (850mg,85%).

d_H ($CDCl_3$) 1.40 9H,s), 3.25 (2H,m), 3.32 (1H,d,J=16.5Hz), 3.50 (1H,d,J=16.5Hz), 4.40 (2H,m), 4.80 (1H,dd,J=6.9,7.2Hz), 6.75 (2H,m), 7.26 (16H,m).

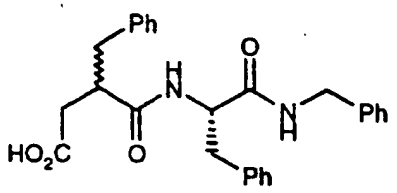
c) N-(2-(R/S)-Benzyl-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-benzylamide



A solution of N-(2-benzylidene-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-benzylamide (790mg,1.58mmol) in ethanol (50ml) was hydrogenated at atmospheric pressure for 4.5h. The reaction was filtered, evaporated under reduced pressure and the residue chromatographed on silica (chloroform-methanol 100:1) to give the desired material as a white solid and a 1:1 mixture of diastereoisomers (660mg,83%) mp 95-100°C.

d_H ($CDCl_3$) 1.25 + 1.41 (9H,2 x s), 2.3-3.5 (7H,m), 4.05-4.70 (2H,m), 4.60-4.80 (1H,m), 5.33-6.02 (2H,m), 6.7-7.4 (15H).

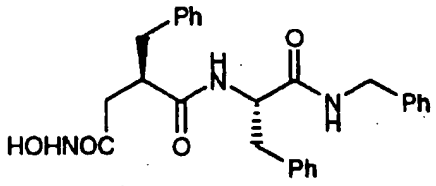
d) N-(2-(R/S)-Benzyl-4-hydroxysuccinyl)-(S)-phenylalanine-N'-benzylamide



A solution of N-(2-(R/S)-benzyl-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-benzylamide (650mg,1.3mmol) in dichloromethane (15ml) was treated with trifluoroacetic acid (15ml) and stirred at 20°C for 4h. The reaction was evaporated and co-evaporated with toluene. Trituration of the residue with ether afforded the acid as a white solid and a 1:1 mixture of diastereoisomers (470mg,81%) mp172-5°C.

d_H [$(CD_3)_2SO$] 2.01-3.03 (7H,m), [4.22 (d,J=5.8Hz) + 4.27 (d, J=5.4Hz), total 2H], 4.53 (1H,m), 7.02-7.31 (15H,m), [8.18 (t,J=5.8Hz) + 8.27 (t,J=5.9Hz, total 1H], [8.22 (d,J=8.3Hz) + 8.35 (d,J=8.5Hz), total 1H], 12.1 (1H,br s,CO_2H).

e) N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-benzylamide

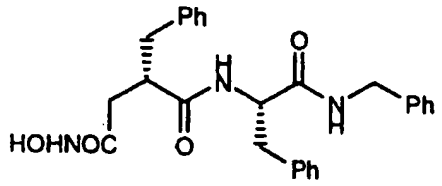


A solution of N-(2-(R/S)-benzyl-4-hydroxysuccinyl)-(S)-phenylalanine-N'-benzylamide as a 1:1 mixture of diastereoisomers (250mg,0.56mmol), diisopropylethylamine (0.2ml,1.12mmol) and O-trimethylsilylhydroxylamine (300mg,2.8mmol) in dichloromethane (10ml) was cooled to 0°C and treated with bromo-tris-pyrrolidinophosphonium hexafluorophosphate (PyBroP) (160mg,0.67mmol) under argon. The reaction was stirred for 1h at 0°C and 16h at 20°C. The organic layer was washed with brine (2x10ml), dried ($MgSO_4$) and evaporated to a sticky solid. This residue was subjected to preparative HPLC

using a VYDAC protein and peptide C_{18} reverse-phase column, eluting with a 1:1 mixture of 0.1%TFA in H_2O : 70% acetonitrile in 0.1% TFA in H_2O at 20ml min^{-1}.
The first eluting diastereoisomer (9.7min) was shown to be N-(2-(R)-benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-benzylamide (50mg,19%) mp 195-7°C.

d_H [$(CD_3)_2SO$] 1.88 (1H,dd,J=6.9,14.9Hz), 2.09 (1H,dd,J=7.5,14.9Hz), 2.72-3.05 (5H,m), 4.21 (2H,m), 4.47 (1H,m), 7.23 (15H,m), 8.18 (2H,m), 8.70 (1H,br s), 10.35 (1H,br s).

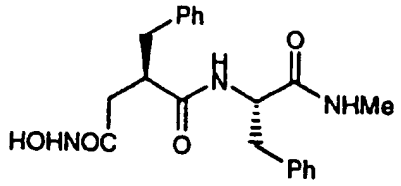
The second eluting diastereoisomer (12.5min) was shown to be N-(2-(S)-benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-benzylamide (30mg,12%) mp 203-5°C.



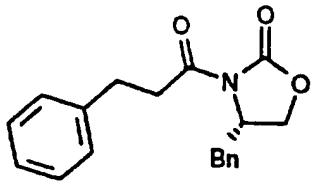
d_H [$(CD_3)_2SO$] 1.92 (1H,dd,J=6.1,15.1Hz), 2.23 (1H,dd,J=8.8,15.0Hz) , 2.35-2.75 (3H,m), 2.98 (2H,m), 4.27 (2H,m), 4.45 (1H,m), 7.21 (15H,m), 8.33 (1H,d,J=8.5Hz), 8.45 (1H,m), 8.71 (1H,s), 10.42 (1H,s).

Example 2

N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-methylamide

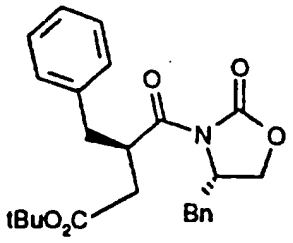


a) 4-(S)-Benzyl-3-(3-phenylpropionyl)-2-oxazolidinone



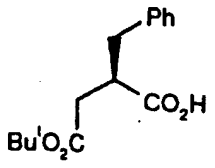
To a solution of 4-(S)-benzyl-2-oxazolidinone (23.9g, 135mmol) in tetrahydrofuran (250ml) at -65°C was added dropwise n-BuLi (1.6M in hexanes 101ml, 178mmol). The resultant orange solution was stirred for 30 minutes then a solution of 3-phenylpropionyl chloride (25g, 148mmol) in tetrahydrofuran (40ml) was added dropwise. After 1.5h the reaction was poured into ice-cold 2M hydrochloric acid (200ml) and extracted with ethyl acetate (3x150ml). The combined organic extracts were washed with 2M hydrochloric acid (150ml), sodium hydrogen carbonate solution (150ml) and brine (100ml). After drying ($MgSO_4$) and evaporation the residue was crystallised from ether to give the title compound as white needles mp 109-111°C. $d_H(CDCl_3)$ 2.75 (1H,dd,J=13.5,10Hz), 2.96-3.07(2H,m);3.17-3.38 (3H,m), 4.14 (1H,s), 4.16 (1H,d,J=2), 4.60-4.70 (1H,m), 7.15-7.35 (10H,m).

b) 4-(S)-Benzyl-3-(2-[tert-butoxycarbonylmethyl]-3-phenyl)propionyl-2-oxazolidinone



To a solution of 1,1,1-3,3,3-hexamethyldisilazane (9.6ml, 45mmol) in tetrahydrofuran (60ml) at -20°C was added dropwise n-BuLi (1.6M in hexanes, 28.3ml, 45mmol). After 45 minutes the solution was cooled to -75°C and a solution of 4-(S)-benzyl-3-(3-phenylpropionyl)-2-oxazolidinone (10g, 32mmol) in tetrahydrofuran (60ml) was added dropwise. The solution was stirred for 1h then allowed to warm to -50°C over the next hour. After cooling to -75°C tert-butyl bromoacetate (7.8ml, 48mmol) in tetrahydrofuran (50ml) was added dropwise and the solution stirred for 1h prior to warming to -20°C over a further 2.5h. The reaction was then poured into saturated ammonium chloride solution (100ml) and extracted with ether (3x100ml). The combined organic extracts were washed with 2M hydrochloric acid (50ml), sodium hydrogen carbonate solution (50ml) and brine (50ml). After drying ($MgSO_4$) and evaporation the yellow oil was triturated with ether/hexane (1:4) to yield the title compound as a white solid (12.3g, 90%) mp 111.5-112.5°C. $d_H(CDCl_3)$ 1.4 (9H,s), 2.37 (1H,dd,J=17,4Hz), 2.64 (1H,dd,J=13,9Hz), 2.72 (1H,dd,J=14,10Hz), 2.85 (1H,dd,J=17,11Hz), 3.01 (1H,dd,J=13,6Hz), 3.31 (1H,dd,J=13.5,3Hz), 3.93 (1H,t,J=6Hz), 4.07 (1H,dd,J=9,2.5Hz), 4.44-4.57 (2H,m), 7.18-7.37 (10H,m).

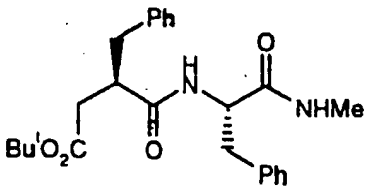
c) 2-(R)-Benzyl-4-tert-butoxysuccinic acid



A solution of 4-(S)-benzyl-3-(2-[tert-butoxycarbonylmethyl]-3-phenyl)propionyl-2-oxazolidinone (5.5g,13mmol) in 3:1 THF/water (200ml) was treated with 27.5% hydrogen peroxide (9.65ml,78mmol) at 0°C and stirred for 10min before the addition of a solution of lithium hydroxide (1.1g,26mmol) in water (100ml). The reaction was stirred for 30min at 0°C and the excess peroxide destroyed by the addition of 1.5M sodium sulphite solution (11g,87mmol) in water (58ml). The solution was buffered to pH 9 with sodium hydrogen carbonate and the THF evaporated. The residue was partitioned between water (300ml) and dichloromethane (300ml) and the aqueous layer washed with dichloromethane (2x200ml). The aqueous layer was acidified with 1M HCl and extracted with ethyl acetate (3x200ml). Drying of the organic layer ($MgSO_4$), filtration and evaporation gave the required acid as a clear oil (3.4g,100%).

$d_H(CDCl_3)$ 1.42 (9H,s), 2.35 (1H,dd,J=4.7,16.8Hz), 2.56 (1H, dd, J=8.7,16.8Hz), 2.77 (1H,dd,J=10.4,15.4Hz), 3.09 (2H,m), 7.17-7.34 (5H,m), acid proton not observed.

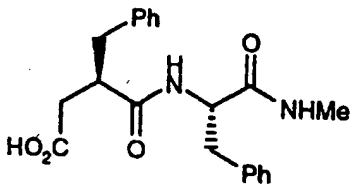
d) N-(2-(R)-Benzyl-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-methylamide



2-(R)-Benzyl-4-tert-butoxysuccinic acid (1g,3.78mmol), (S)-phenylalanine-N'-methylamide hydrochloride (812mg,3.78mmol), N,N-diisopropylethylamine (0.66ml,3.78mmol), 1-hydroxybenzotriazole hydrate (919mg,6.8mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (797mg,4.16mmol) in DMF (70ml) were treated as in example 1 b) to give the title compound as a white solid following recrystallisation from ethyl acetate-hexane (1.15g,72%).

d_H ($CDCl_3$) 1.43 (9H,s), 2.38 (1H,dd,J=4.4,16.8Hz), 2.57 (3H,d,J=4.7Hz), 2.53-2.99 (5H,m) 3.15 (1H,dd,J=5.8,13.8Hz), 4.54 (1H,m), 5.33 (1H,br m), 5.90 (1H,br d,J=8Hz), 7.12-7.31 (10H,m).

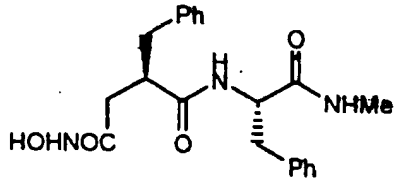
e) N-(2-(R)-Benzyl-4-hydroxysuccinyl)-(S)-phenylalanine-N'-methylamide



N-(2-(R)-Benzyl-4-tert-butoxysuccinyl)-(S)-phenylalanine-N'-methylamide (1.02g,2.41mmol) and TFA (10ml) in dichloromethane (10ml) were treated according to example 1 d) to give the required acid as a white solid (830mg,94%).

d_H [$(CD_3)_2SO$] 2.03 (1H,dd,J=5.5,16.5Hz), 2.33 (1H,dd,J=8.4,16.6Hz), 2.50 (3H,d,J=4.6Hz), 2.56 (1H,m), 2.75-2.99 (4H,m), 4.39 (1H,m), 7.14-7.27 (10H,m), 7.47 (1H,m), 8.17 (1H,d,J=8.2Hz), 12.07 (1H,br s).

f) N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-methylamide

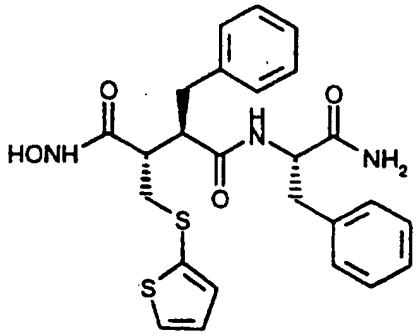


A solution of N-(2-(R)-benzyl-4-hydroxysuccinyl)-(S)-phenylalanine-N'-methylamide (0.8g,2.17mmol) in THF (50ml) was cooled to -20°C and treated with N-methylmorpholine (0.29ml,2.61mmol) and isobutylchloroformate (0.34ml,2.61mmol) and the reaction stirred for 1h at -20°C. O-TMS-hydroxylamine (2.2ml,20.9mmol) was added dropwise, the reaction allowed to warm to ambient temperature and stirred for 16h. After that time, the reaction was evaporated and partitioned between ethyl acetate and 10% citric acid. The organic layer was washed with brine and dried ($MgSO_4$). Filtration and evaporation gave a solid which was recrystallised from ethyl acetate-methanol to give the title compound as a white solid (300mg,36%) mp 188-190°C.

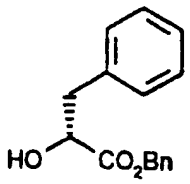
d_H [$(CD_3)_2SO$] 1.91 (1H,dd,J=7.2,15.0Hz), 2.06 (1H,dd,J=7.2,15.0Hz), 2.50 (4H,m), 2.75 (2H,m), 2.95 (2H,m), 4.31 (1H,m), 7.06-7.27 (10H,m), 7.38 (1H,m), 8.11 (1H,d,J=8.2Hz), 8.73 (1H,s), 10.4 (1H,s).

Example 3

N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalaninamide.



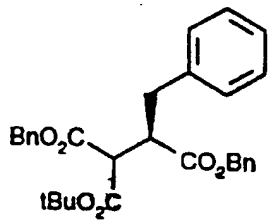
a) Benzyl 3-(R)-phenyllactate.



A solution of 3-(R)-phenyllactic acid (20.381g, 123mmol) in THF (160ml) was treated with triethylamine (23.5ml, 17.02g, 168.5mmol) and benzyl bromide (16.2ml, 23.18g, 135.3mmol) and the mixture stirred at reflux for 1h. After cooling the precipitate was removed by filtration and the filtrate evaporated. The residue was taken up in ethyl acetate, washed with saturated sodium hydrogen carbonate, 1M HCl, brine, and dried ($MgSO_4$). Filtration and evaporation gave the title compound as a yellow oil (24.086g, 76%).

d_H [$(CD_3)_2SO$] 2.84 (1H, dd, J=7.97, 13.75 Hz), 2.97 (1H, dd, J=5.5, 13.75 Hz), 4.26-4.34 (1H, m), 5.09 (2H, s), 5.62 (1H, d, J=6.32 Hz), 7.16-7.43 (10H, m)

b) Dibenzyl 2-(R)-benzyl-3-tert-butoxycarbonylsuccinate



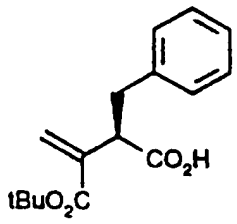
Sodium hydride (60% dispersion in mineral oil, 3.37g, 84.3mmol) was added to a solution of benzyl tert-butyl malonate (21.1g, 84.4mmol) in DMF (165ml) at 0°C. The solution was allowed to warm to room temperature and stirred, under argon, for 1h.

Triflic anhydride (21.85ml, 36.6g, 130.2 mmol) was added in one portion to a solution of benzyl 3-(R)-phenyllactate(24g, 93.75mmol) and pyridine (7.09ml, 86.8mmol) in dichloromethane (220ml) at 0°C. The solution was stirred for 1h under argon. The mixture was washed with ice cold water, brine, dried ($MgSO_4$), and filtered.

The filtrate was added to the DMF solution of malonate at 0°C and the solution allowed to warm to ambient temperature and stirred for 16h. Following evaporation, the residue was dissolved in ethyl acetate, washed with saturated sodium hydrogen carbonate solution, 1M HCl, brine and dried ($MgSO_4$). Filtration and evaporation gave a residue which was chromatographed on silica (5 % ethyl acetate-hexane) to give the title compound as an oil (24.67g, 60%).

d_H ($CDCl_3$) 1.35 (9H, d, J=11.55 Hz) 2.84-3.02(2H, m), 3.38-3.48 (1H, m), 3.68 (1H, dd, J=3.7, 9.5 Hz), 4.86-5.19 (4H, m), 7.03-7.37 (15H, m)

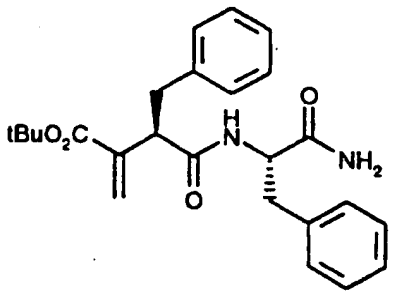
c) 2-(R)-Benzyl-4-tert-butoxy-3-ethenylsuccinic acid.



A solution of dibenzyl 2-(R)-benzyl-3-tert-butoxycarbonylsuccinate(14.64g, 30mmol) in isopropyl alcohol (90ml) was treated with 10% Pd/C and hydrogenated at atmospheric pressure. The reaction was filtered and the filtrate treated with piperidine (8.91ml, 90mmol) and formaldehyde (37%, 36.63ml, 450mmol) and stirred at ambient temperature overnight. Following evaporation the residue was dissolved in ethyl acetate, washed with 1M HCl, brine, and dried ($MgSO_4$). Filtration and evaporation gave the title compound as a colourless oil (7.17g, 87%).

d_H ($CDCl_3$) 1.48 (9H, s), 2.96 (1H, dd, J=8.25,14.02 Hz), 3.26 (1H, dd, J=7.98, 14.02 Hz), 3.75 (1H, t, J=7.98 Hz), 5.55 (1H, s), 6.23 (1H, s), 7.14-7.30 (5H, m), acid proton not seen.

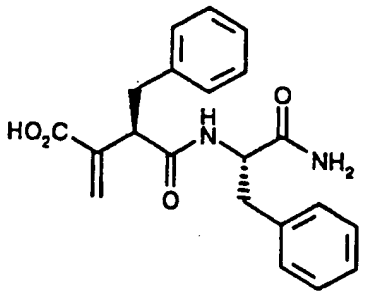
d) N-(2-(R)-Benzyl-4-tert-butoxy-3-ethenylsuccinyl)-(S)-phenylalaninamide.



A solution of 2-(R)-benzyl-4-tert-butoxy-3-ethenylsuccinic acid (7.083g, 25.63mmol), (S)-phenylalaninamide hydrochloride (4.68g,23.3mmol), diisopropylethylamine (3.97ml, 23.3mmol) and 1-hydroxybenzotriazole hydrate (7.16g, 46.4mmol) in DMF (80ml) was treated at 0°C with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.35g, 27.96mmol) and the reaction stirred for 1h at 0°C and 16h at ambient temperature. Following evaporation, the residue was dissolved in dichloromethane, washed with 10% citric acid, saturated sodium hydrogen bicarbonate, and dried ($MgSO_4$). Filtration and evaporation gave a residue which was chromatographed on silica (gradient elution hexane-ethyl acetate 7:3 to 3:7) to give the title compound as a white solid (5.83g, 60%).

d_H [$(CD_3)_2SO$] 1.39 (9H, s), 2.68-2.79 (2H, m), 2.92-3.05 (2H, m), 3.72 (1H, t, J=7.42 Hz), 4.38-4.47 (1H, m), 5.44 (1H, s), 5.94 (1H, s), 7.03-7.25 (12H, m), 7.90 (1H, d, J=8.25 Hz).

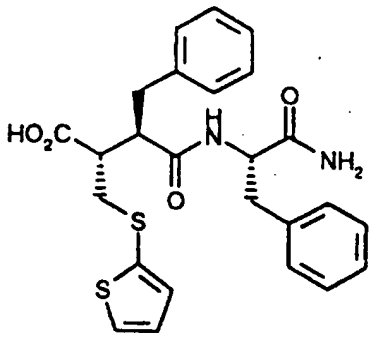
e) N-(2-(R)-Benzyl-4-hydroxy-3-ethenylsuccinyl)-(S)-phenylalaninamide.



A solution of N-(2-(R)-benzyl-4-tert-butoxy-3-ethenylsuccinyl)-(S)-phenylalaninamide (2.11g, 5mmol) in dichloromethane (50ml) was treated with trifluoroacetic acid (50ml) and stirred at ambient temperature for 3h. The reaction was evaporated and co-evaporated with toluene. Trituration of the residue with ether afforded the title compound as a white solid (1.59g, 87%).

d_H [$(CD_3)_2SO$] 2.70-2.78 (2H, m), 2.92-3.05 (2H, m), 3.77 (1H, t, J=7.42 Hz), 4.36-4.44 (1H,m), 5.48 (1H, s), 6.02 (1H, s), 7.04-7.24 (12H, m), 7.87 (1H, d, J=8.53 Hz), 12.59 (1H, br. s).

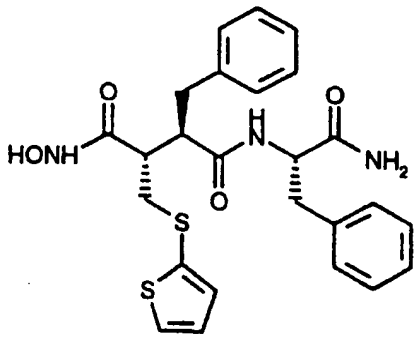
f) N-(2-(R)-Benzyl-4-hydroxy-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalaninamide.



A solution of N-(2-(R)-benzyl-4-hydroxy-3-ethenylsuccinyl)-(S)-phenylalaninamide(1.48g, 3.9mmol) in methanol (20ml) was treated with 2-mercaptothiophene (5.07ml) at ambient temperature and heated under reflux under argon for 18h. The reaction was evaporated and co-evaporated with toluene. Trituration of the residue with ether afforded the title compound as a white solid (1.63g, 86%).

d_H [$(CD_3)_2SO$] 1.95 (1H, dd, J=3.03, 12.92 Hz), 2.28-2.75 (6H, m), 2.98 (1H, dd, J=4, 13.5 Hz), 4.32-4.41 (1H, m), 6.32 (1H, s), 6.91-7.27 (13H, m), 7.60 (1H, dd, J=1.37, 5.22 Hz), 8.19 (1H, d, J=8.53Hz), 12.70 (1H, br. s)

g) N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalaninamide.

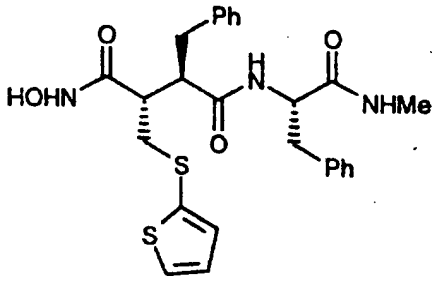


A solution of N-(2-(R)-benzyl-4-hydroxy-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalaninamide(1.445g, 3mmol) in THF (25ml) and DMF (10ml), at -20°C, under argon, was treated with N-methylmorpholine (0.42ml, 3.6mmol) and isobutylchloroformate (0.48ml, 489mg, 3.6mmol). After stirring at -20°C for 1h the solution was treated dropwise with O-trimethylsilylhydroxylamine (2.6ml, 2.53g, 21mmol) and stirred at ambient temperature for 16h. Following evaporation the residue was dissolved in ethyl acetate and washed with 10% citric acid, brine and dried ($MgSO_4$). After filtration, evaporation and trituration with ether the solid was heated in ethyl acetate and the residue filtered to give (after repetition of this a number of times) the title compound as a white solid (25mg).

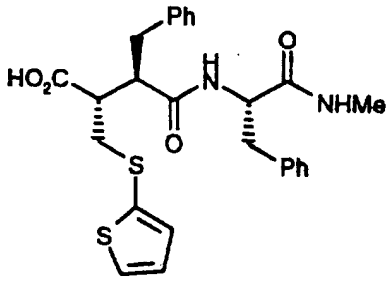
d_H [$(CD_3)_2SO$] 1.81 (1H, dd, J=2.3, 12.5 Hz), 2.25-2.66 (6H, m), 2.97 (1H, dd, J=3.7, 13.5 Hz), 4.25-4.33 (1H, m), 5.89 (1H, s), 6.85 (1H, s), 6.96-7.24 (12H, m), 7.56 (1H, dd, J=1.2,5.4Hz), 8.13 (1H, d, J=8.52 Hz), 8.98(1H, s), 10.71 (1H, s).

Example 4

N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalanine-N'-methylamide



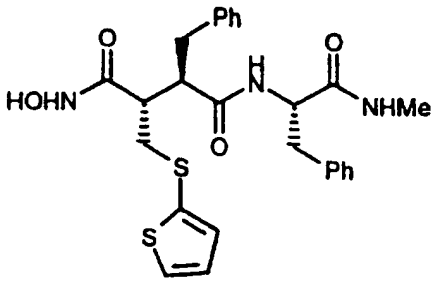
a) N-(2-(R)-Benzyl -4-hydroxy-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalanine-N'-methylamide



Prepared according to Example 3f but using N-(2-(R)-benzyl-4-hydroxy-3-ethenylsuccinyl)-(S)-phenylalanine-N'-methylamide (200mg,0.53mmol), to give the title compound as a white solid (100mg,38%)

d_H [$(CD_3)_2SO$] 1.94 (1H,dd,J=3.2,12.8Hz), 2.28-2.72 (6H,m), 2.44 (3H,d,J=4.7Hz), 2.93 (1H,dd,J=4.1,13.5Hz), 4.37 (1H,m), 6.79 (1H,m), 7.01-7.27 (12H,m), 7.60 (1H,dd,J=1.5,5.1Hz), 8.23 (1H,d,J=8.8Hz), 12.7 (1H,br s).

b) N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalanine-N'-methylamide



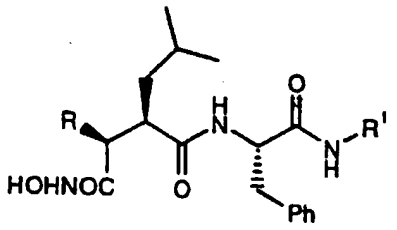
Prepared according to Example 3g affording the title compound as a white solid (35%).

d_H [$(CD_3)_2SO$] 1.83 (1H,d,J=10.5Hz), 2.30 (1H,m), 2.39 (3H,d,J=4.7Hz), 2.50-2.63 (5H,m), 2.94 (1H,dd,J=4.1,13.7Hz), 4.33 (1H,m), 6.45 (1H,m), 7.00 (4H,m), 7.17 (8H,m), 7.56 (1H,dd,J=1.4,5.2Hz), 8.20 (1H,d,J=8.2Hz), 8.97 (1H,br s), 10.70 (1H,br s).

Activity Data

Compound	CD23 proteinase inhibition % at 1uM	Collagenase inhibition IC50 uM
Example 3	105	0.93
Example 4	85	0.37
Comparative Example*	96	0.005

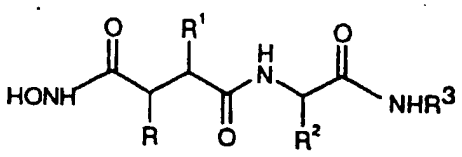
*The comparative example was Example 2 of WO90/05719, the compound of formula:



wherein R is CH_2S-(2-thienyl) and R^1 is methyl.

Claims

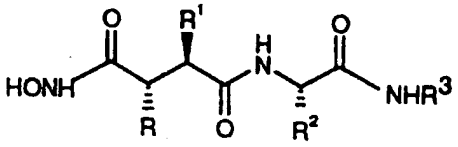
1. A compound of formula (I):



(I)

wherein R and R3 are each independently hydrogen, alkyl, alkenyl, alkynyl or aryl; R1 is arylmethyl; and R2 is alkyl, alkenyl, aryl, cycloalkyl or cycloalkenyl, or a pharmaceutically acceptable derivative thereof.

2. A compound of formula (IA):



(1A)

wherein R to R3 are as defined in claim 1, or a pharmaceutically acceptable derivative thereof.

3. A compound according to claim 1 or 2 wherein R is hydrogen or methyl, optionally substituted by arylthio or heterocyclylthio; and/or R1 is a benzyl group; and/or R2 is a benzyl group; and/or R3 is hydrogen, methyl or benzyl.

4. A compound selected from the group consisting of:
N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-benzylamide,
N-(2-(R)-Benzyl-4-hydroxyaminosuccinyl)-(S)-phenylalanine-N'-methylamide,
N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalaninamide, and
N-(2-(R)-Benzyl-4-hydroxyamino-3-(S)-[2-thienothiomethyl]succinyl)-(S)-phenylalanine-N'-methylamide.

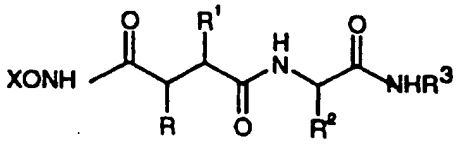
5. Use of a compound according to any preceding claim for the production of a medicament for the treatment or prophylaxis of disorders in which the overproduction of s-CD23 is implicated.

6. A method for the treatment or prophylaxis of disorders in which the overproduction of s-CD23 is implicated, which method comprises the administration of a compound according to any one of claims 1 to 4, to a human or non-human mammal in need thereof.

7. A pharmaceutical composition for the treatment or prophylaxis of disorders in which the overproduction of s-CD23 is implicated which comprises a compound according to any one of claims 1 to 4, and optionally a pharmaceutically acceptable carrier.

8. A process for preparing a compound according to any one of claims 1 to 4, which process comprises:

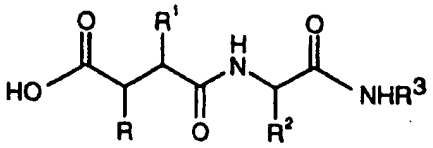
(a) deprotecting a compound of formula (II):



(II)

wherein R to R3 are as defined in claim 1, and X is a protecting group such as benzyl or trimethylsilyl or

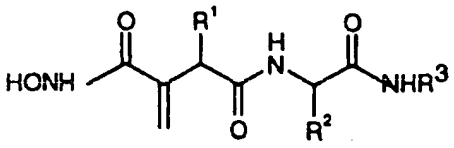
(b) reacting a compound of formula (III):



(III)

wherein R to R3 are as defined in claim 1, with hydroxylamine or a salt thereof, or

(c) reacting a compound of formula (IV):



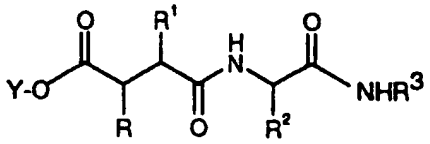
(IV)

wherein R1 to R3 are as defined in claim 1, with a thiol to give a compound of formula (I) wherein R is methyl substituted by alkylthio, arylthio, aralkylthio, or heterocyclylthio, or

(d) converting a compound of formula (I) to a different compound of formula (I).

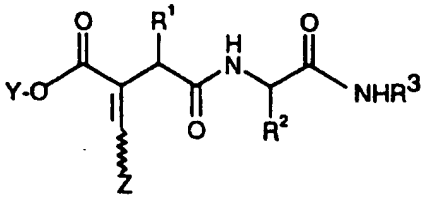
9. A compound of formula (II), (III), or (IV) as defined in claim 8.

10. A compound of formula (V):



(V)

wherein R to R3 are as defined in claim 1, and Y is a protecting group such as t-butyl; or a compound of formula (VI):



(VI)

wherein R1 to R3 and Y are as defined hereinabove, and Z is a group such that ZCH_2 is R;

INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/EP 97/00196

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D333/34 C07C259/06 A61K31/38 A61K31/185

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 05719 A (BRITISH BIO TECHNOLOGY) 31 May 1990 cited in the application see page 46; claims see page 16-20 ---	1-10
P,X	WO 96 26918 A (BRITISH BIOTECH PHARM ;FLOYD CHRISTOPHER DAVID (GB); WHITTAKER MAR) 6 September 1996 see page 24; claims see page 15 ---	1-10
X	EP 0 214 639 A (SEARLE & CO) 18 March 1987 see page 8; example 13 --- -/--	1,2,8-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 1997	30.05.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Sánchez García, J.M.

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INTERNATIONAL SEARCH REPORT

Int nal Application No
PCT/EP 97/00196

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MOLECULAR PHARMACOLOGY, vol. 30, no. 4, 1986, pages 338-344, XP000671433 P. CHEROT ET AL: "Enkephalin-degrading dipeptidylaminopeptidase: Characterization of the active site and selective inhibition" see page 341; example 6 ---	1,2
X	BIORGANIC & MEDICINAL CHEMISTRY LETTERS, vol. 4, no. 23, 1994, pages 2741-2746, XP000671434 J.R. PORTER ET AL: "Potent and selective inhibitors of gelatinase-a 1. Hydoxamic acid derivatives." see table 1 -----	1,2

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

...nformation on patent family members

Int ...nal Application No
PCT/EP 97/00196

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		DE 68914687 T	08-09-94
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		AU 6240886 A	12-03-87
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		DK 169029 B	01-08-94
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